EFFECT OF ADRENAL PREPARATIONS ON TUMOR GROWTH

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Evidence has been accumulating in the literature for an influence of the adrenal cortex on malignant growth. Beck and Diller (1946) and Diller *et al.* (1948) reported definite degenerative changes and decrease in size of transplanted mouse sarcoma 37 following intraperitoneal injections of 0.5 to 1.0 cc. of a potent adrenal extract.

It seemed of interest to us, therefore, to investigate the effects on sarcoma 37 of more extended treatment with adrenal extract and with certain fractions obtainable from the adrenal. The effect of desoxycorticosterone acetate (DCA) was observed for comparison only since this substance is not an adrenal hormone.

Methods

All animals used in this study were adult albino mice of the Carworth Farms strain, 10 to 12 weeks of age. A number of animals bearing sarcoma 37 were obtained from the Lankenau Hospital Research Institute through the courtesy of Dr. Irene Corey Diller. The tumor was kept growing in our own laboratory by transplanation into female mice every 10 to 14 days. This material provided the stock tumor for all experiments. Since sarcoma 37 sometimes shows spontaneous regression, experimental and control groups were always implanted and run simultaneously in the following manner, after the method of Dr. Diller (Diller, 1947). Viable tumors were removed from the host aseptically, divided into fragments and drawn into a number 15 trocar for implantation. Inoculations were made subcutaneously into the ventral surface of the recipient, the skin having previously been defurred and cleaned with 70 per cent alcohol. Transplants were routinely allowed a seven day development period. The tumor-bearing mice were then divided into three groups on the basis of tumor size as determined by palpation, *i.e.,* large, medium and small tumors. When animals for the experimental and control series were chosen, equal numbers were taken from each of these groups. In most cases, except as noted below, they were injected twice daily with the various preparations for periods ranging from 5 to 7 days. The age of the tumors at the time the animals were cancelled was 15–17 days. On the day following the injection period experimental and control groups were killed with ether. After being measured with calipers along two axes, one in a right angled plane to the other, the tumors were carefully removed and the living tissue separated from necrotic areas with the aid of a binocular loupe. The living tissue was weighed in a glass-stoppered weighing bottle either from individuals or from experimental groups. In some cases as noted, dry weight was also determined. The following preparations were used in experiments as designated: 1. Upjohn's whole adrenal extract, 1 cc. = 60 g. tissue; 2. Whole adrenal extract prepared in our own laboratory, 1 cc. = 60 g. tis-

sue; 3. DCA [1] in oil, 1 cc. = 5 mg.; 3a. DAC in 15% alcohol, 1 cc. = 5 mg.; 4. sodium hormone in 10% alcohol prepared in our laboratory by Dr. J. S. Thatcher, 1 cc. = 300 g. tissue; 5. 17-hydroxy-11-dehydrocorticosterone [2] in 10% alcohol, 1 cc. = 0.6 mg.; 6. 11-dehydrocorticosterone [3] in 10% alcohol, 0.6 mg./cc.

Results

Effect of whole adrenal extract

The results with adrenal extract, though variable, were so pronounced as to leave little doubt of its power to inhibit the growth of sarcoma 37 in female mice when adequate dosage was used (see Table I). Thus, one injection of 0.5 cc.

Table I

Effect of whole adrenal extract on tumor growth

Group treatment	No. of mice	Dosage daily, cc.	Wt. of living tumor tissue				Degree of necrosis			
			Wet wt. avg.	% change	Dry wt. avg.	% change	0	+	++	+++
1 Adrenal Ext. A	12 ♀	.1 4×	.151	+104	.122	+198				
Controls	13 ♀		.069		.041					
2 Ext. A	15 ♀	.3 2×	.137	+37	.029	+27				
Controls	15 ♀		.100		.023					
3 Ext. B	17 ♀	.4 2×	.076	−42	.016	−41	0	5	6	6
Controls	20 ♀		.131		.027		1	7	8	1
4 Ext. B	9 ♂	.4 2×	.154	+208	.030	+123				
Controls	8 ♂		.050		.013					
5 Ext. C	7 ♀	*	.262	0			0	3	4	0
C	7 ♀	.1 1×	.213	−18			0	3	2	2
C	7 ♀	.2 1×	.031	−88			0	0	2	5
Controls	7 ♀		.260				0	5	2	0
6 Ext. C	11 ♂	.1 2×	.189	−28			2	3	4	2
Controls	10 ♂		.264				1	2	7	0

*—1 inj. only, 0.5 cc. 5 days before cancellation.
A—our own.
B—Upjohn, lot 1.
C—Upjohn, lot 2.
0—none.
+—slight.
++—moderate.
+++—complete.

(Group 5) five days before cancellation had no effect on tumor growth; 0.1 cc. injected daily for 5 days (Group 6) prior to cancellation caused a negligible (18 per cent) reduction in amount of viable tumor tissue present considering the variability, while 0.2 cc. injected daily for 5 days (Group 6) brought about almost complete disappearance of viable tissue as compared with controls. At the dosage level of 0.2 cc. daily for five days, there was a 28% reduction in amount of viable tumor tissue

[1] Kindly supplied by Roche-Organon, Inc.
[2] Supplied through the courtesy of Dr. E. C. Kendall.
[3] Kindly supplied by Merck and Co.

in male mice (Group 6). This effect is remarkable since in males this tumor has been found notably resistant to adrenal preparations (Diller *et al.*, 1948). Another batch of adrenal extract was found to have no inhibiting effect at daily dosage levels of 0.4 and 0.6 cc., but at a level of 0.8 cc. daily the inhibition of sarcoma 37 in female mice was marked (Table I, Group 3), amounting to 42%. This extract at the same dosage level caused an actual increase in tumor growth in male mice. It is interesting to note that extract seems to reduce tumor size not so much by inhibiting growth as by accentuating the processes leading to necrosis. The data on degree of necrosis show this. In the group of 7 animals treated with 0.2 cc. extract C daily (Group 5), two showed a moderate degree of necrosis (++) while in five necrosis was complete (+++). In the controls of this series two exhibited a moderate degree of necrosis (++) while in five necrosis was very slight (+). Again in Group 3 about the same relationship obtains as to degree of necrosis observed, there being 6 out of 17 tumors completely necrotic (+++) in the extract-treated group while only one tumor out of 20 in the control series showed total necrosis. This observation is further substantiated by tumor measurements on this group at the time of killing. The average measurements on the control tumors were 91 × 127 mm. while those on the experimental group were 98 × 125 mm. or approximately identical. However, on weighing the living tissue from each group, the average value for the treated animals was found to be 0.076 g., 40 per cent below that of the control animals (0.131 g.).

Effect of sodium-retaining substances

Because of the small amount of sodium hormone available, this treatment was tried in only one group of 15 animals (Table II, Group 5). It resulted in a questionably significant reduction (26 per cent) in living tumor tissue when compared to the control group. As in the case of whole adrenal extract the reduction appeared to be due to more rapid progress of necrosis rather than to direct

TABLE II

Effect of sodium-retaining substances on tumor growth

Group treatment	No. of mice	Dosage daily, cc.	Wt. of living tumor tissue				Degree of necrosis			
			Wet wt. avg.	% change	Dry wt. avg.	% change	0	+	++	+++
1 DCA in oil	6 ♀	*	.172	+60			0	2	2	2
Controls	6 ♀		.108				0	1	1	4
2 DCA in oil	13 ♀	.1 4×	.243	+254			10	3	0	0
Controls	13 ♀		.069				9	3	1	0
3 DCA in alcohol	15 ♀	.3 2×	.175	+75	.036	+55	2	6	4	1
Controls	15 ♀		.100		.023		5	7	2	1
4 DCA in oil	11 ♂	.1 2×	.312	+18			2	5	4	0
DCA in alcohol	11 ♂	.1 2×	.272	+3			0	4	6	1
Controls	10 ♂		.264				1	2	7	0
5 Sodium factor	15 ♀	.1 2×	.085	−26	.018	−22	1	4	7	3
Controls	15 ♀		.115		.023		9	3	3	0

* 0.5 cc. subcut., 0.5 cc. intraper. 7 days before cancellation.

TABLE III

Effect of some crystalline gluconeogenic substances on tumor growth

Treatment	No. of mice	Dosage	Wt. of living tumor tissue	
			Wet avg.	% change
17-hydroxy-11-dehydrocorticosterone (cortisone)	7 ♀	1 inj. 300 γ in 10% alc. 5 days before cancellation	.428	+65
11-dehydrocorticosterone	7 ♀	1 inj. 300 γ in 10% alc. 5 days before cancellation	.385	+48
Controls	7 ♀		.260	

inhibition of growth. It will be noted in the table that three sodium hormone-treated animals exhibited complete necrosis (+++) while seven showed a moderate amount (++) and only one animal showed none, while in nine animals of the control group no necrosis was observed and none of the others showed marked necrosis. This rather striking difference in necrosis in favor of the sodium hormone-treated animals further strengthens the questionably significant data on tumor tissue weights. The synthetic sodium-retaining substance, DCA, had the opposite effect on tumor growth. All dosages employed produced marked increases in tumor growth in female animals; in the male insignificant increases were observed (Group 4, Table II). DCA in oil appeared to be a more potent tumor growth stimulant (254 per cent increase) than DCA in alcohol (75 per cent increase), probably because in alcohol the substance is more rapidly absorbed and destroyed. It will be noted (Group 3) that the increase in living tumor tissue is significantly more on the wet weight basis than on that of dry weight. This is to be expected from the known action of desoxycorticosterone acetate in increasing body water generally.

Effect of crystalline gluconeogenic substances

The amounts of crystalline gluconeogenic compounds available at the time these observations were made seriously limited our study of these substances. Seven female mice were injected subcutaneously with 300 gamma each of 17-hydroxy-11-dehydrocorticosterone (cortisone) and an equal number of mice with the same amounts of 11-dehydrocorticosterone. All were cancelled five days later. Both substances definitely increased tumor growth as compared to controls (Table III), and there was no significant difference between them. In the case of the cortisone-treated animals there seemed to be some increase in amount of necrosis over that found in control animals. This might be due to the cortisone treatment or possibly to larger tumors more rapidly outgrowing their blood supply. From the data available there is no means of differentiating these two factors.

DISCUSSION

There is some evidence that a low level of adrenal cortical secretion is associated with tumor growth and that a high level is inhibitory to such growth. Thus, Haven and Asheworth (1950) found steroids in the adrenal markedly decreased in rats bearing Walker tumor 256 even though the gland weights in these animals were

significantly increased, and Dobriner and co-workers (1950) found urinary ketosteroid excretion low in patients with neoplastic disease. Haven *et al.* (1949) observed somewhat increased steroid content of the adrenals of rats in which Walker tumor 256 either did not take, or in which it took and regressed.

Of the direct, inhibitory effect of adrenal grafts, adrenal tissue extracts, synthetic adrenal hormones or synthetic products possessing some properties similar to adrenal hormones, most observations seem to be on lymphoid tumors, or leukemia (Law and Speirs, 1947; Murphy and Sturm, 1944, 1950; Woolley, 1950). These authors all observed at least temporary regression. Their results are in line with the well known observations of involution of the thymus and lymph nodes when additional cortical hormones are administered to normal animals. Of such effects on sarcoma 37, the only evidence of which we are aware aside from our own (Table I) is that of Beck and Diller (1946) and Diller *et al.* (1948) using small amounts (0.5 to 1.5 cc.) of Upjohn's adrenal extract as noted in the introduction. In our hands one injection of 0.5 cc. Upjohn's extract had no effect on growth (Table I, Group 5) as measured by weight of viable tissue five days after the treatment. There are two possible reasons for this difference. First, we were using a different batch of extract and second, we cancelled our animals five days after the injection while Diller *et al.* (1948) noted the greatest incidence of tumor disappearance 17–35 days after treatment.

Our results using cortisone (Table III) are at variance with those reported in the literature in that with sarcoma 37 it produced an actual increase rather than decrease in tumor growth. Heilman and Kendall (1944) working with lymphoid tumors, Higgins *et al.* (1950) using transplanted rhabdomyosarcoma and Burchenal *et al.* (1950) observing leukemic mice obtained results in the direction of inhibition or regression. All of these workers used massive doses of cortisone.

Both large and small doses of DCA (Table II) also stimulated the growth of sarcoma 37. The work of Lipschütz and Zañartu (1942) showed this substance to be anti-fibromatogenic in female guinea pigs treated with estrogens. On the other hand, Kupperman and Greenblatt (1946), working with transplanted sarcoma in rats, obtained results comparable to ours (Table II), namely an enhanced growth of the tumor in animals treated with DCA. It will be noted (Table II, Group 3) that the increase in tumor tissue is considerably greater on the wet (75 per cent) than on the dry weight (55 per cent) basis. This is in line with observations in the literature on other tissues taken from animals treated with DCA (Zuckerman *et al.*, 1950). Although the sodium hormone acts like DCA in causing sodium retention it is unlike this substance in other respects (Thatcher and Hartman, 1946, Hartman *et al.*, 1939). It is interesting to note that the two compounds also differ in their effect on sarcoma 37; DCA caused a marked increase in growth of the tumor while sodium hormone caused a questionably significant reduction in amount of viable tissue present at autopsy and more rapid progress of necrosis than occurred in controls. The sodium hormone used on these animals was not a pure substance. It was purified only to the extent that it contained no gluconeogenic hormone and therefore presumably no compounds with oxygen in the C_{11} position. It is noteworthy that such an adrenal fraction possesses some power to inhibit the growth of sarcoma 37 and to promote necrosis. There is, in the finding of Dobriner *et al.* (1950), that steroids with oxygen in the C_{11} position are *not* decreased in the urine of patients with neoplastic disease while other steroids are, a further suggestion that

other adrenal hormones are more important in this condition than those with gluconeogenic properties. Further investigation of the sodium hormone fraction is planned.

Summary

1. The effects of adrenal extract, sodium hormone, DCA, 17-hydroxy-11-dehydrocorticosterone (cortisone) and 11-dehydrocorticosterone on transplantable mouse sarcoma 37 have been investigated. More than 250 animals were used.

2. Injection of adrenal extract for 5 days caused as high as 88% decrease in viable tissue and marked necrosis of the tumor as compared to controls.

3. Sodium hormone caused a questionably significant decrease in viable tissue (26%) and marked increase in necrosis.

4. DCA increased tumor growth in all doses employed.

5. Both cortisone and 11-dehydrocorticosterone in single relatively small doses (300 γ) enhanced the growth of sarcoma 37.

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